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BACILLARY WHITE DIARRHEA AND RELATED DISEASES OF CHICKENS

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Bacillary White Diarrhea and Related Diseases of Chickens

By P. R. EDWARDS and F. E. HULL

Because of the unusual manner of transmission of bacillary white diarrhea of poultry, the etiology and cycle of infection of which have long been known, measures of control of the disease must be applied to the breeding flock. Therefore a sure and expeditious means of detecting the infection in living fowls must be of the greatest practical value. The agglutination test has been used very successfully for this purpose and practically all attempts to eradicate the disease have been dependent on it. The dependability of the method has been questioned in some quarters however, and new, more rapid methods have been proposed. The purpose of this investigation was to test the accuracy of the procedures in use heretofore and of some of those recently suggested to supersede them.

At times infections occur in chicks hatched from blood-tested stock that have not been exposed to *Salmonella pullorum*. Such occurrences, unless the chicks are definitely shown not to be infected with *S. pullorum*, are likely to cause an unfavorable reaction toward the agglutination test. These outbreaks may be due to other bacilli of the Salmonella group. An infection of this kind has been encountered and a description of the disease together with the identification of the causative organism is included in this report.

METHODS

Thruout the course of the work reported here the method

of conducting the agglutionation test has remained constant. The antigen employed was composed of five strains of *S. pullorum,* all of known agglutinability. It was made up to a density of 0.5 on the MacFarland nephelometer. The saline solution used to dilute the antigen contained 0.85 percent of sodium chloride and 0.5 percent of phenol. Two cubic centimeters of antigen were used. Two dilutions were employed, 1 to 40 and 1 to 80. Duplicate tests were set up with antigen to which 1 percent of normal sodium hydroxide solution had been added just before use. After the serum had been put into the antigen tubes they were thoroly shaken and incubated at 37° C. for 48 hours before reading. Agglutination in either dilution was considered indicative of infection.

The addition of sodium hydroxide to the antigen has been of great value in preventing cloudiness in the tests. The results obtained with the antigen to which sodium hydroxide had been added with the antigen without sodium hydroxide were in nearly every instance the same. With very weakly agglutinating serums the antigen with sodium hydroxide usually gave slightly stronger reactions and the statement of Mallmann[1], that it is more sensitive, is probably correct. However, in routine testing, the difference in reactions obtained with these two antigens except for the elimination of cloudy tests, was negligible.

In post mortem examinations the entire ovary was removed. Both large and small ova were examined for gross lesions. All ova showing any abnormality were cultured. In culturing, the ovum to be examined was seared with a heated spatula, the ovisac punctured with a sterile knife and the contents of the ovum streaked on agar slants. After the ovary had been examined the other internal organs were examined for pathological changes. Where any lesions were found, cultures were made. A search was made thruout the body for cysts and where any were found the contents were cultured. Where any other pathological changes were noted in the internal organs they were examined bacteriologically.

[1] Mallmann, W. L., Jour. Amer. Vet. Med. Assoc., 71, 1927, 600-606.

Colonies developing on the tubes inoculated were examined morphologically. All colonies which yielded Gram-negative rods were planted in dextrose, maltose, dulcitol and lactose broth. The broth was composed of 1 percent peptone and 0.3 percent meat extract. The reaction was adjusted to pH 7.2, Andrade's indicator and the fermentable substances were added in amounts of 1 percent. The fermentable substances were not heated but were sterilized separately by filteration and added to the previously sterilized broth. After the addition of the fermentable substances, all tubes were incubated at 37°C. for 72 hours to insure sterility. Those cultures which produced acid in dextrose and failed to ferment maltose, dulcitol and lactose, after incubation for seven days at 37°C., were subjected to the action of agglutinating serum derived from the injection of rabbits with cultures of *S. pullorum.* The cultures which exhibited characteristic morphological, cultural and fermentative characters and which were acted upon by the agglutinating serum are recorded as *S. pullorum.*

THE INTRADERMAL TEST IN THE DETECTION OF BACILLARY WHITE DIARRHEA

Several attempts have been made to evolve a satisfactory intradermal test for *S. pullorum* infection in fowls. Ward and Gallagher,[2] in 1917, proposed an antigen consisting of a thirty-day broth culture of *S. pullorum* concentrated to one-fifth of its original volume. Scherago and Benson,[3] in 1919, reported unsuccessful results with the antigen of Ward and Gallagher. Not only did they find a lack of specificity in the test, but 85 percent of the birds tested in this manner became positive reactors to the agglutination test.

Fuller,[4] in 1923, proposed an antigen which was essentially the same as that employed by Ward and Gallagher. There is no record of a comparison of the agglutination and intradermal tests in this work. It is based upon chick mortality before and after testing and upon macroscopic pathology of the ovaries of

[2] Ward, A. R., and Gallagher, B. A., U. S. Dept. Agr. Bul. 517, 1917.
[3] Scherago, M., and Benson, J. B., Cornell Vet., **9**, 1919, 111-117.
[4] Fuller, J. W., Rpt. N. Y. State Vet. Col., 1922-1923, 59-61.

certain birds which were autopsied. Fuller concluded that (a) the intradermal test detects a large percentage of infected individuals but does not detect them all in heavily infected flocks and (b) that only a very small percentage of the non-carriers react to the test.

Broerman,[5] in 1927, described an antigen for intradermal testing which was prepared by a different method from that of Ward and Gallagher. In this method *S. pullorum* was grown for 48 hours on agar, the organisms washed off with sterile distilled water and centrifuged. They were washed four times in this manner, carbolized, and allowed to stand until killed. This suspension was then diluted with 0.5 percent phenol to a density of tube one on the McFarland nephelometer. Comparative agglutination and intradermal tests were run on two flocks. In one flock sixteen birds reacted to one or both tests. On a retest, six birds gave doubful or positive reactions to the intradermal test. Four gave positive or doubtful reactions to the agglutination test. All of the six birds showed macroscopic ovarian lesions. No attempts to culture the ovaries are reported nor are the methods used in the agglutination test recorded. Broerman concluded that the intradermal test is of value in detecting infected fowls.

Graham and Tunnicliff[6] recently reported on comparative studies of the agglutination and intradermal tests with postmortem examination of a number of reacting fowls.

In testing 158 birds they found 48.7 percent positive to agglutination and 85.6 percent positive to the intradermal test. Ovarian lesions were observed in 91.7 percent of the birds at autopsy. Cultures were taken from the ovaries of seventeen birds which were positive to agglutination. Ten of these yielded *S. pullorum.* Twenty-three birds giving positive intradermal reactions were cultured. *S. pullorum* was recovered from the ovaries of ten.

Bushnell[7] described an antigen for intradermal testing

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[5] Broerman, A., Jour. Amer. Vet. Med. Assoc., **70**, 1927, 597-604.
[6] Graham, R., and Tunnicliff, E. A., Jour. Amer. Vet. Med. Assoc., **70**, 1927, 612-628.
[7] Bushnell, L. D., Jour. Infect. Diseases, **43**, 1928, 60-66.

which consisted of the precipitate formed on heating saline washings of *S. pullorum*. The results obtained with this product agreed much more closely with the agglutination test than did the results obtained thru the use of commercial intradermal antigen. However, this method was not considered sufficiently accurate to replace the agglutination method in the diagnosis of bacillary white diarrhea.

Experimental Work

The experiments reported here were made with a commercial intradermal antigen marketed under the name "pullorin," and with several products prepared in this laboratory. The work with the commercial prepartion has been more extensive than that with the other preparations and will be considered first.

The method of preparation of this commercial product, "pullorin," is not known. The literature of the manufacturers states that their best results in preparing the agent have been obtained by "precipitating the active principles of certain strains of the S. pullora organisms by methanol." Pullorin injected intradermally is said to indicate the infection by producing an edematous swelling in birds carrying *S. pullorum*.

Methods

In making the intradermal injections, a syringe graduated to .04 cc was used with a 26-gage intradermal needle. One-twenty-fifth of a cubic centimeter was injected into each fowl. The injection was made intradermally, as near the ventral border of the wattle as possible. The pullorin was dissolved in sterile physiologic saline solution, furnished by the laboratory making the product. It was allowed to stand for one hour to permit the insoluble portion to subside. The insoluble material was avoided in filling the syringe.

Three different lots of pullorin, as evidenced by the serial numbers, were used in these tests. There was a marked lack of uniformity in these different lots of pullorin. This lack of uni-

formity in the product was evidenced by the difference in the amount of insoluble material and the color of the solution.

The tests were read at the end of twenty-four hours. All soft, edematous swellings were considered positive. Hard swellings were read as negative.

Results

The results of the agglutination and the pullorin tests are record in table 1. In this table the birds are divided into four groups, on the basis of their reactions to the two tests. These groups are composed of (a) birds giving a positive reaction to the intradermal test, which failed to react to the agglutination test; (b) birds reacting positively to the agglutination tests, which failed to react to the intradermal test; (c) birds giving positive reactions to both tests; (d) birds giving negative reactions to both tests. The figures are given separately for each flock tested and as a total.

From table 1, it can be seen that there is a very poor correlation between the pulorin and agglutination tests. Many of the birds reacting positively to the agglutination test gave negative intradermal reactions, while many birds giving positive intradermal reactions were negative to agglutination. Only 47.6 percent of the birds which were positive to the agglutination test were reactors to the intradermal test. The number of agglutination reactors which were detected by pullorin varied in the different flocks from 28.6 percent to 62.5 percent. The percentage of agglutination reactors detected by pullorin is apparently independent of the percentage of infection in the flocks. This variability is difficult to explain. The same condition was encountered by Graham and Tunnicliff.[6] The technic of the agglutination and intradermal tests was kept as nearly constant as possible. This variance in the percentage of agglutination reactors giving positive intradermal tests may possibly be due to the antigen employed in the intradermal injections. The physical properties of the product varied markedly; it is possible that

[6] Graham, R., and Tunnicliff, E. A., Jour. Amer. Vet. Med. Assoc., 70, 1927, 612-628.

TABLE 1.—REACTIONS TO AGGLUTINATION AND INTRADERMAL TESTS

Flock No.	1		2		3		4		5		Total	
Number of birds in flock	75		91		74		87		328		655	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Agglutination negative, pullorin positive	7	9.3	11	12.2	8	10.8	24	27.6	33	10.0	83	12.8
Agglutination positive, pullorin negative	22	29.2	15	16.7	3	4.0	15	17.2	22	6.7	77	11.1
Agglutination positive, pullorin positive	23	30.8	20	22.2	5	6.8	6	6.9	16	4.8	70	10.6
Agglutination negative, pullorin negative	23	30.8	45	49.9	58	78.4	42	48.3	257	78.5	425	65.5
Positive to agglutination, per cent	60.0		38.9		10.8		24.1		11.5		21.7	
Positive to pullorin per cent	40.1		34.4		17.6		34.5		14.8		23.4	
Agglutination reactors detected by pullorin per cent	51.1		57.1		62.5		28.6		42.1		47.6	
Pullorin reactors detected by agglutination per cent	76.7		64.5		38.5		20.0		32.6		45.7	
Pullorin reactors negative to agglutination per cent	23.3		35.5		61.5		80.0		67.4		54.3	

the potency of the pullorin varied also. The same agglutination antigen was used in all the tests, so that the variability is not traceable to this factor.

Another serious discrepancy between the two tests appears in table 1. This is the large number of birds reacting to the intradermal test which gave negative reactions to the agglutination test. Only 45.7 percent of the birds which reacted to the pullorin tests were also positive to the agglutination test, while 83 birds, or 54.3 percent, of the pullorin-positive fowls were negative to the agglutination test. The post mortem examinations indicated that a very large percentage of the birds which gave positive intradermal tests and negative agglutination tests were not infected with *S. pullorum*. If this be true, the removal of such birds from breeding flocks would mean a serious loss to the poultryman. The possibility of these birds being carriers of *S. pullorum* will be discussed later.

Post-Mortem Examinations

On post mortem examination of the fowls, some abnormal conditions were found in the ovaries which were apparently not due to *S. pullorum* infection. While it is not proposed to go into a detailed discussion of the pathology of the avian ovary some of these conditions may be mentioned.

One of the most common abnormalities in the ovaries of hens which are negative to the agglutination test is the presence of cysts which result from the closing of the stigmal rupture in the calyx. Normally, when the ovary of a hen that has been laying intensively for some time is examined, numerous ruptured calices are found. These vary in size from a calyx large enough to hold an ovum which is ready to be discharged, down to very small bodies the size of a pinhead. The larger sacs are those which have enclosed ova which have been very recently discharged; the smaller ones are apparently the calices of ova discharged some time before.

When the ovum is discharged into the oviduct the calyx is left as a ruptured sac, pendant from the ovary. Apparently

when the physiological processes continue uninterruptedly, these calices degenerate and decrease in size until they are no longer noticeable upon macroscopic examination. At times, however, a pathological change takes place to interrupt this degenerative process. For some reason the rupture along the stigma of the calyx becomes closed and fluid is collected in the cavity. The fluid found in these cysts varies in color from a light yellow to a

TABLE 2.—POST-MORTEM EXAMINATIONS

Band Number	Pullorin Test	Agglutination Test	Cultural Results*	MACROSCOPIC PATHOLOGY
2463	—	—	—	Hen in laying condition. Apparently normal
H229	—	—	—	Ovary apparently normal except for two transudation cysts
D76	+	—	—	Hen in laying condition. Ovary apparently normal
2460	+	—	—	Two transudation cysts
110	+	—	—	One blood clot and one transudation cyst
D80	+	—	—	Small ova only. Apparently normal
H228	+	—	—	Ovary apparently normal
46	+	—	—	Cyst on oviduct. One slightly angular ovum
H216	+	—	—	Ovary normal except for one transudation cyst
14	+	—	—	Ovary meaty, tumorous. Hen suffering with myeloid leukemia
H159	+	—	—	Several flabby ova, slightly discolored
H150	+	—	—	Three large flabby ova
H219	+	—	—	Small cyst on oviduct; transudation cysts
H87	+	—	—	Three transudation cysts in ovary
H72	+	—	—	Ovary apparently normal
H87	+	—	—	Two small ova discolored; one transudation cyst
H32	+	—	—	streptococcus recovered
H222	+	—	—	Two very small, slightly discolored bodies in ovary
H35	+	—	—	Ovary apparently normal
H246	+	—	—	Ovary apparently normal
2473	+	+	+	Practically all ova cystic, angular, discolored
152	+	+	+	Many angular, discolored ova
H223	+	+	+	One small, angular discolored ovum
H352	+	+	+	Several angular, discolored ova
H313	+	+	+	50% of ova discolored and angular. Cyst on oviduct

* + S. pullorum recovered. — S. pullorum not recovered.

TABLE 2.—POST-MORTEM EXAMINATIONS—(Continued)

Band Number	Pullorin Test	Agglutination Test	Cultural Results*	MACROSCOPIC PATHOLOGY
H582	—	+	+	Many angular, discolored ova
H579	—	+	+	Few very small angular, discolored ova
886	—	+	+	Cockrel. Pericarditis, epicarditis, pericardial sac filled with pus. S. pallorum from the pericardial fluid.
B396	—	+	+	Many angular discolored ova
H414	—	+	C	Several small, angular, discolored ova
987	—	+	+	Many ova, hard, discolored, angular
97	—	+	+	50% of ova angular and discolored
H175	—	+	+	Several very small, angular, discolored ova
18	—	+	+	Pericarditis. Caseous exudate in pericardial sac. Angular, discolored ova
H136	—	+	+	One abnormal ovum. Vessels of membrane congested. Filled with thick, white fluid
H102	—	+	+	Many small, angular, discolored ova. Large ova apparently normal
H244	—	+	—	Several small ova, angular and discolored. Large ova apparently normal
127	—	+	+	Necrotic areas on liver, hemorrhages on heart, ovary apparently normal
G15	—	+	+	Cockrel. Slight pericarditis, cyst on brachial artery, cyst on vas deferens
104	—	+	+	Many angular, discolored ova
H257	—	+	+	Ovary very small. One cyst filled with purulent fluid. Large mass of organized exudate in peritoneal cavity. Concretion in oviduct

C = Contamination.

very dark, brownish red, probably depending upon the amount of blood present in the fluid. The result of such a condition might be called a retention cyst or a transudation cyst. Such names are not usually applied to cystic conditions of the ovary. Cysts occurring in the ovary are usually spoken of as follicular cysts or corpus luteum cysts. The condition described here is more or less analogous to the corpus luteum cysts of the higher animals. However, one hesitates to apply such a term to the

avian ovary. Gage[8] has mentioned retention cysts in the ovary of the fowl. While he does not define this term, it is not thought that he refers to the condition described here, but to degenerated ova. In order to avoid confusion of terms we will speak of these bodies as transudation cysts.

In the birds which were positive to the agglutination test and from which *S. pullorum* was recovered, diseased ova were usually found. These ova were similar to those described by Rettger,[9] in 1914, as ova harboring *S. pullorum*. In the post-mortem reports these are described as diseased or cystic ova.

In table 2 the results of the post-mortem examinations are given. In this table are included the reaction of the hen to the agglutination test, the reaction to the pullorin test, the recovery or non-recovery of *S. pullorum* on cultures, and a brief description of any pathological changes in the ovary and other internal organs.

The most significant fact brought out in table 2 is the high percentage of birds giving positive agglutination tests from which *S. pullorum* was recovered at autopsy. Twenty-one birds, giving positive agglutination tests, were cultured. *S. pullorum* was recovered from nineteen birds, the cultures from one bird were contaminated, and the cultures from one bird were negative. These figures are in close accord with those of Jones,[10] reported in 1913, who recovered *S. pullorum* from twenty of twenty-one birds giving positive agglutination tests. Sixteen of these birds from which *S. pullorum* was recovered had given negative pullorin tests.

Seventeen birds giving positive pullorin tests and negative agglutination tests were autopsied. In no instance was *S. pullorum* recovered from these birds. In one bird of this group (H32) a streptococcus was recovered from an abnormal ovum. We have found several cases in our work at this laboratory that yielded a streptococcus from ovaries which looked very much

[8] Gage, G. E., Paige, B. H., and Hyland, H. W., Mass. Agr. Exp. Sta. Bul. 148, 1914.
[9] Rettger, L. F., Kirkpatrick, W. F., and Jones, R. E., Conn. Storrs. Agr. Exp. Sta. Bul. 77, 1914.
[10] Jones, F. S., Jour. Med. Research, 27, 1913, 481-495.

like *S. pullorum* ovaries. Of nineteen hens giving negative agglutination tests and from which *S. pullorum* was not recovered, only seven possessed ovaries which showed no macroscopic pathological changes. Graham and Tunnicliff[6] report ovarian lesions in a large number of birds giving negative agglutination tests and from which *S. pullorum* could not be recovered. We believe that these pathological changes are not due to *S pullorum* infection. A large number of the abnormalities found in birds of this class are the formations which we have designated as transudation cysts. We have never recovered *S. pullorum* from one of these bodies.

While the birds giving negative agglutination tests and positive pullorin tests exhibit abnormal ovaries in many instances, the abnormalities are quite different from those found in birds giving positive agglutination tests. Transudation cysts and blood clots predominate in the birds giving positive intradermal and negative agglutination tests; angular, discolored ova are found in the large majority of the birds giving positive agglutination tests. *S. pullorum* can be cultivated from these angular, discolored ova in a high percentage of the cases.

From the results of the post-mortem examinations it seems that the agglutination test is a much more accurate index of *S. pullorum* than is the pullorin test. Only 47.6 percent of the birds reacting to the agglutination test reacted to the pullorin test. On the basis of the pullorin tests many infected birds would have been left in the flocks. On the other hand, 12.8 percent of the total number of birds tested were positive to pullorin and negative to agglutination. The post-mortem examinations indicate that the large majority of these birds were not carriers of *S. pollorum,* but that the reactions in these birds were non-specific.

Several products prepared in this laboratory were tried in an effort to devise a satisfactory method of intradermal testing. These products were as follows:

[6] Graham, R., and Tunnicliff, E. A., Jour. Amer. Vet. Med. Assoc., 70, 1927, 612-628.

1. Agar cultures of *S. pullorum* suspended in distilled water as recommended by Broerman.[5]

2. Filtrates of alkaline saline suspensions of *S. pullorum* which had been frozen and thawed a number of times.

3. Saline suspensions of *S. pullorum* which had been cultivated on Melick's medium.

4. The filtrates of Melicks medium in which *S. pullorum* had been cultivated for 30 days at 37°C.

The number of birds tested with each was not large but was sufficient to show that these products are not of practical value. The results obtained by the use of these preparations were not perceptibly better than those obtained with the commercial product.

Tho no satisfactory method seems to have been devised whereby bacilary white diarrhea may be detected intradermally, the results of certain investigators are sufficiently promising to warrant further work on the problem. It is to be hoped that a practical method may be evolved, but until this has been accomplished the routine use of the intradermal test for the detection of *S. pullorum* infection should be discouraged.

THE SLIDE AGGLUTINATION TEST IN THE DETECTION OF BACILLIARY WHITE DIARRHEA

The slide or rapid agglutination test as applied to the detection of bacillary white diarrhea by Runnels, Coon, Farley and Thorp[11] is being widely used. Several reports concerning this test have recently appeared. Results obtained at the California Experiment Station[12] indicate that the slide method equals the tube test in efficiency. Stafseth and Thorp[13] found that different antigens varied in sensitivity. However, when a suitable antigen was used the results obtained by the slide test agreed closely with the tube test. Bushnell[7] concluded that "the rapid slide microscopic agglutination test is probably as efficient in detecting reactive fowls as the tube method and can be used as

[11] Runnells, R. A., Coon, C. J., Farley, H., and Thorp, F., Jour. Amer. Vet. Med. Assoc., 70, 1927, 660-662.
[12] California Agricultural Experiment Station Report, 1927, 95-99.
[13] Stafseth, H. J., and Thorp, F., Jour. Amer. Vet. Med. Assoc., 72, 1928, 745-756.

a substitute for the latter. Bushnell and Brandley[14] found the slide test very effective in the detection of reactors. Work at the Wisconsin Experiment Station[15] indicates that the slide method is as efficient as the tube method.

Since the results obtained in this laboratory by the use of this method are not in full agreement with the observations cited above it seems worth while to present them here.

Experimental Work

The results reported were obtained by the repeated testing of a flock of hens. This flock has varied in number from 50 to 125. The birds were tested at monthly intervals. Both slide tests and tube tests have been performed.

The antigen used in the slide tests was prepared according to recommendations of Runnels, Coon, Farley and Thorp.[11] Three strains of *S. pullorum* were used in the preparation of the antigen. These strains were the same as those used in making antigen for the tube test and were known to furnish satisfactory antigen for the agglutination test. The concentration of the antigen used in the slide tests was 50 times the concentration used in the tube tests. In performing the slide tests 0.02 cc of serum were placed in contact with 0.02 cc of antigen and thoroly mixed. After a brief period of warming the slide the readings were taken. The serums which gave negative reactions at this reading were subjected to additional stirring and a second reading was taken. Altho Bushnell and Brandley[14] warn that continued stirring tends to create positive reactions which cannot be confirmed by the tube method, tests made here show that additional stirring of initial negative reactions has resulted in a much better correlation than when this procedure is not used.

Using the two methods, 1150 samples of serum were tested. The results are as follows:

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[14] Bushnell, L. D., and Brandley, C. A., Jour. Amer. Vet. Med. Assoc., 73, 1928, 844-847.

[15] Wisconsin Agricultural Experiment Station Report, 1928.

Tube agglutination positive	slide agglutination positive,	682
	slide agglutination negative,	96
Tube agglutination negative	slide agglutination positive,	12
	slide agglutination negative,	360

The coefficient of association according to the method of Yule is .99. The two methods agree in 90.6 percent of the tests. The above figures seem to indicate that the tube test gives a larger number of reactors than the rapid test.

If the sera of different agglutinative titers are considered, a very interesting correlation is brought out. Below the results obtained with serums of different titers are given separately.

Agglutination complete at 1 to 80	Slide test positive,	582
	Slide test negative,	6
Agglutination complete at 1 to 40 partial but not complete 1 to 80	Slide test positive,	51
	Slide test negative,	18
Complete agglutination at 1 to 40 no agglutination at 1 to 80	Slide test positive,	20
	Slide test negative,	16
Definite but incomplete agglutination at 1 to 40, no agglutination at 1 to 80	Slide test positive,	29
	Slide test negative,	56

From the summary given above it may be seen that when serums giving a complete agglutination at 1 to 80 or higher are tested the agreement obtained with the two methods is nearly 99 percent. However, as the agglutinative titer of the serums declines the percentage of agreement becomes markedly less. In this work the presence of definite agglutination at a dilution of 1 to 40 has been considered indicative of infection. Several of the birds whose serum gave incomplete agglutination at 1 to 40 were examined and found to be carriers of *S. pullorum*. Therefore the application of the tube method seems not to have been too rigid.

It is obvious that the antigens used in the slide tests were not sufficiently sensitive to detect all the infected birds. No explanation is apparent for this lack of sensitivity. Commercial antigens have been compared with the antigens prepared in the laboratory. The latter were found more sensitive than the commercial antigens. If the commercial preparations had been

TABLE 4.—REACTIONS OF HENS TESTED AT MONTHLY INTERVALS

	12-7-27	1-24-28	2-24-28	3-23-28	4-25-28	5-24-28	6-25-28	7-25-28	8-22-28	9-25-28	10-24-28	11-26-28	12-18-28	1-23-29	2-26-29	3-25-29	Autopsy
H259		+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +				S. pullorum
H286		+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +				S. pullorum
H293		+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +				S. pullorum
H310		+ +	+ +	+ +	+ +	+ +	+ +	+ +									S. pullorum
H322		+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +				S. pullorum
H413	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +					S. pullorum
H416	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +					S. pullorum
H421	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +					S. pullorum
H441	+ +																S. pullorum
H454	+ +	+ +	+ +														S. pullorum
H461	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +					S. pullorum
H472	+ +	+ +	+ +	+ +	+ +	+ +	+ +										S. pullorum
H479		+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +				S. pullorum
H484	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +									S. pullorum
H488	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ ±	+ +	+ +					S. pullorum
H489	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +					S. pullorum
H505	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +					S. pullorum
H506	+ +	+ ±	+ ±	± ±	+ ±	+ —	+ —	± ±									Lost
44	+ +	+ +	+ +	+ +	+ +												S. pullorum
2471		+ +	+ +	+ +	+ +	+ +											S. pullorum
2491	+ +	+ +	+ ±	+ +	+ +	+ +	+ +	+ ±	+ ±	+ ±	+ —	+ +	+ +				Negative
2495	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +					S. pullorum
H10				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
H12				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +						S. pullorum
H16				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
H20				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
H28				+ +	+ +	+ +	+ +	+ ±	+ ±	+ —	± ±						S. pullorum
H30				+ +	+ +	+ +	+ +	+ ±	+ ±	+ —	± ±	+ —	+ +				S. pullorum

TABLE 4.—REACTION OF HENS TESTED AT MONTHLY INTERVALS—Continued.

	12-7-27	1-24-28	2-24-28	3-23-28	4-25-28	5-24-28	6-25-28	7-25-28	8-22-28	9-25-28	10-24-28	11-26-28	12-18-28	1-23-29	2-26-29	3-25-29	Autopsy
H31				+ +	+ +	+ +											S. pullorum
H46				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
H68				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +					S. pullorum
H75				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
H76				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
H82				+ +	+ ±	+ ±	+ ±	+ ±	± +	+ ±	± +	+ ±	+ +	+ ±	+ ±	+ +	S. pullorum
320				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
370				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
55				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
92				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +					S. pullorum
N912				+ +	+ +	+ +	+										S. pullorum
169					+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
144					+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
130					+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
146					+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
J768							+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
J997			+ +	+ +	+ +	+ +	+ +	+ +	+	+ ±	+ ±	+ +	+ +	+ +	+ +	+ +	S. pullorum
F100			+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +				S. pullorum
A5903			+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
A5905			+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
A5946			+ +	+ +	+ +	+ +	+ +	+ +									S. pullorum
H610		+ +	+ +	+ +	+ +	+ +	+ +	+ +										Post Mortem Decomposition
H613			+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
H623			+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
H626			+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
H647			+ +	+ +	+ +	+ +	+ +										S. pullorum
H650			+ +	+ ±	+ +	+ ±	+ —	+ +	+ +	+ +	+ —	+ —	+ +	+ +	+ +	+ +	S. pullorum
24			+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum

TABLE 4.—REACTION OF HENS TESTED AT MONTHLY INTERVALS—Continued.

	12-7-27	1-24-28	2-24-28	3-23-28	4-25-28	5-24-28	6-25-28	7-25-28	8-22-28	9-25-28	10-24-28	11-26-28	12-18-28	1-23-29	2-26-29	3-25-29	Autopsy
64				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
79				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
85				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	± +	E. sanguinarium
136				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
153				+ +	+ +	+ +	+ +	+ ±	+ ±	+ —	+ ±	+ +	+ +				S. pullorum
156				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
170				+ +													S. pullorum
227				+ +	+ ±												S. pullorum
241				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	S. pullorum
267				+ +	+ +	+ +	+ +	+ +	+ ±								S. pullorum
J2312			+ +	+ +	+ +	+ +	+ +	+ +	+ +								S. pullorum
J2329			+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ ±	+ ±	+ +	+ +	+ +	+ +		S. pullorum
J2347			+ +	+ +	+ +	+ +	+ +	+ +	+ —	+ ±	+ ±	+ —	+ +	+ +	+ +		S. pullorum
JJ2351			+ +	+ +	+ +	+ +											S. pullorum
J2388			+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +		S. pullorum
J2339				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +		S. pullorum
H197					+ +	+ +	+ +	+ +	+ —	± —	— —	± —	+ +	± —	± —	± —	Negative
H432	+ ±	+ ±	+ ±	+ +	+ +	+ ±	+ ±	+ ±	+ ±	+ —	+ ±	+ ±	+ +	+ ±	+ +	± ±	S. pullorum
347				+ ±	± ±	+ —	+ —	+ —	± ±	+ ±	± —	+ —	+ +	+ —	+ +	+ ±	S. pullorum
H467	+ ±	+ ±	+ —	+ —	± ±	+ —	+ —	± —	+ —	+ —	+ ±	+ ±	+ —				S. pullorum
LWT5				+ ±	+ +	+ +	+ +	± ±	± —	+ —	+ ±	+ ±	+ ±	+ +	+ +	+ +	S. pullorum
A5952		+ ±	± ±	± ±	+ ±	+ ±	+ —	± ±	± ±	± —	± —						Lost
A608		+ ±	+ ±	± ±	+ ±	+ +	+ —	+ ±	± —	+ ±	+ ±						Lost
27				+ ±	+ ±	+ +	± ±	+ ±	± ±	± ±	+ ±	+ ±	+ +	+ +	+ ±	± —	S. pullorum
69				+ ±	+ +	+ +	+ +	+ +	+ +	± ±	+ —	+ +	+ +	+ —	+ ±	+ —	S. pullorum
167				+ ±	+ +	+ +	+ +	+ ±									S. pullorum
851		+ ±	+ ±	+ ±	± ±	± ±											S. pullorum

TABLE 4.—REACTION OF HENS TESTED AT MONTHLY INTERVALS—Continued.

	12-7-27	1-24-28	2-24-28	3-23-28	4-25-28	5-24-28	6-25-28	7-25-28	8-22-28	9-25-28	10-24-28	11-26-28	12-18-28	1-23-29	2-23-29	3-25-29	Autopsy
41				± —	+ +	+ ±	+ —	+ ±	+ +	+ +	+ +						S. pullorum
H422			± ±	+ —	± —	± ±	± ±	± ±	± —	± —	± —	± —	+ —	± —	± —	± —	Negative
344				± ±	± —	± ±	± —	± ±	— —	± —	± —	— —	± —	— —	± ±		Lost
165				± ±	± —	± ±	± ±	± ±	— —	± —	— —	— —	± —	± —	± ±	± —	Negative
H462	± ± —	+ +	± ±	± —	± —	± ±	± ±	± ±	± —	± —	± ±	± —	± —	— —	± ±		S. pullorum
H612	± —	± —	± ±	± —	± ±	± —	± ±	± —	± —	± ±	± ±	± —	± ±	+ ±	+ —		S. pullorum
316	+ +	+ —	+ +	+ +	+ +	+ +	± —	± ±	+ —	+ —	+ +	+ +	+ ±	+ +			S. pullorum
H107				± ±	± ±	± ±	± ±	± —	± —	± ±	± —	± +	+ ±	± ±	± ±		S. pullorum
J706				± ±	+ ±	± ±	± —	± ±	— —	— ±	— —	± +	+ +	± ?			Streptococcus

— — No agglutination at 1 to 40 or 1 to 80.

± — Partial agglutination at 1 to 40, no agglutination at 1 to 80.

± ± Partial agglutination at 1 to 40 and 1 to 80.

+ — Complete agglutination at 1 to 40, no agglutination at 1 to 80.

+ + Complete agglutination at 1 to 40 and 1 to 80.

used thruout the course of the investigation the degree of correlation would have been even less than we have found it. Both fresh antigen and antigen which had been kept in the ice box for some time were used. The results obtained with the fresh antigen were not perceptibly better than those obtained with older preparations.

Mention should be made of the 12 serums which were positive to the slide tests and negative to the tube tests. Repeated retesting of these birds by both methods has demonstrated that in 11 instances the birds were really negative reactors. One of these 12 tests represented a bird which was later positive by both methods.

Summary

The antigens prepared in this laboratory for use in the slide agglutination test and the commercial antigens examined were found lacking in sensitiveness. The slide test has been found quite satisfactory in detecting birds whose serums caused complete agglutination in a dilution of 1 to 80 or higher. The slide test has been found less efficient than the tube test in detecting reactors whose serums agglutinated in lower dilutions only.

THE PRECIPITATION TEST IN THE DIAGNOSIS OF BACILLARY WHITE DIARRHEA

In the application of the agglutination test for the detection of bacillary white diarrhea many partial or doubtful reactions are encountered. At times it is extremely difficult to decide whether a reaction is slightly positive or whether it is in reality negative. If a supplementary test could be developed to aid in the diagnosis of such cases it would be very useful. Bushnell[7] used the complement fixation test in conjunction with the agglutination test. The results obtained thru the use of the two tests agreed closely. However, it was found that a number of the serums tested were anti-complementary. Also the complement fixation reaction is too laborious and time consuming to be used in testing large numbers of animals.

The precipitin test is more simple and lends itself more

readily to routine work than the complement fixation test. If a satisfactory antigen could be prepared this method might prove a valuable supplement to the agglutination test in testing doubtful birds.

Several methods have been used in the preparation of precipitin antigens from *S. pullorum*. This included the freezing and thawing of dense suspensions of the bacilli and the use of the supernatant fluid after centrifuging. Extraction of the bacilli with varying percentages of alcohol was also tried. However, the most satisfactory antigens which we have obtained are the saline washings from heavy suspensions of *S. pullorum*. In preparing these antigens the bacilli were washed from agar slants or flats with a small amount of physiological saline solution to which 0.5% of phenol had been added. The resulting dense suspension was filtered thru cheesecloth to remove small pieces of agar and stored in the icebox from one to six months. The suspension was then centrifuged to clearness and the supernatant fluid used as antigen. One percent of normal sodium hydroxide solution was added to the antigen just before use to prevent clouding.

In setting up the test four tubes were employed. Into each of these tubes 0.1cc of the serum to be tested was pipetted. To the first three tubes antigen was added in the amounts of 0.5cc, 0.2cc and 0.1cc. The fourth tube served as a serum control. Normal saline containing one percent of normal sodium hydroxide solution was added where needed to make up the volume of liquid in the tubes to 0.6cc. The tests were incubated at 56° for one hour and a reading taken. They were then placed in the ice box overnight and again read the following morning. In most cases there was no change in the tests between the time of the first and second readings. Any cloudiness developing in the tubes containing antigen which was absent in the serum control was recorded as a positive reaction. Using an incubation period of one hour at 56° cloudiness was rarely encountered in the control tube. After standing overnight nonspecific clouding sometimes occurred.

The amount of precipitation occurring in the tubes varied from the formation of a heavy disk precipitate to a barely perceptible clouding. Whether the amount of precipitate formed is dependent upon the agglutinative titer of the serum cannot be stated, since no agglutination tests were performed at a higher dilution than 1:80. It is true, however, that serums causing complete agglutination at 1.80 as a rule gave a larger amount of precipitate than did the serums of lower titers.

Following are the results obtained in testing 169 birds by the agglutination and precipitin tests:

Agglutination positive	Precipitin positive	103=60.9%
	Precipitin negative	17=10.0%
Agglutination negative	Precipitin positive	6= 3.5%
	Precipitin negative	43=25.4%
Coefficient of Association, 095+		

The two methods agree in 86.3 percent of the tests.

If the serums of different agglutinative titers be considered separately the following results are obtained.

Agglutination complete at 1 to 80	Precipitin positive	87
	Precipitin negative	4
Agglutination complete at 1 to 40	Precipitin positive	3
	Precipitin negative	2
Agglutination complete at 1 to 40 negative at 1-80	Precipitin positive	4
	Precipitin negative	4
Agglutination partial at 1 to 40 negative at 1-80	Precipitin positive	9
	Precipitin negative	7

As would be expected, the precipitin test was more efficient in detecting infected individuals having a relatively high agglutinative titer. As the agglutinative titer declined the agreement between the two tests decreased.

Repeated testing and post-mortem examination of the birds giving positive agglutination and negative precipitin tests has proved that these birds were almost without exception carriers of *S. pullorum*. The birds giving positive precipitin and negative agglutination tests were, as far as could be determined, non-infected individuals.

While the precipitin test as employed here is apparently

of some value in the detection of the disease it is obvious that it is not so sensitive as the agglutination test. If a more sensitive antigen could be prepared the method would probably be of some value in testing partial or doubtful birds. It is not subject to the long incubation usually given the agglutination test and the danger of bacterial contamination is avoided. Until a more sensitive antigen is developed, however, this test will not be of practical value in the diagnosis of bacillary white diarrhea.

THE COMPARATIVE TESTS OF SERUM IN DIFFERENT LABORATORIES

Practically every one of the serological tests which have been widely used in the diagnosis of human and animal diseases has been criticized by some investigators. Recently these criticisms have been based upon data obtained by sending portions of samples of serum to several laboratories for diagnosis. The results of these procedures have not always been encouraging. Among the diagnostic tests which have been subjected to such analysis are the Wasserman reaction and the agglutination test for the detection of bovine infectious abortion. Gilbert and Langworhy[16] repored upon a number of samples of serum sent to different laboratories to be tested for syphilis. The results of these tests were quite variable; only 66 percent correlation was obtained. The agglutination test for bovine infectious abortion has met severe criticism from various workers. Yet today these two serological tests are accepted as being of great value in the detection of disease and their application for the determination of infection is very videly practiced.

During the past year Beach and Merick[17] published the results obtained by sending samples of chicken serum to six laboratories to be tested for bacillary white diarrhea. The results of this comparison were far from encouraging. The serums of 38 hens were sent to the six laboratories. In only five

[16] Gilbert, R., and Langworthy, V., Paper presented at meeting of Am. Pub. Health Assoc., 1927.

[17] Beach, B. A., and Merrick, A. C., Amer. Poultry Jour., 58, 1927, 404-405.

samples were the reports identical. Other similar tests reported by the same investigators agreed no more closely.

Tests were conducted in a similar manner to those of Beach and Merrick, as follows: Twenty-four hens which were being used in experimental work were bled from the wing vein, the blood placed in the icebox overnight and the serum drawn off the following morning. The serums were divided into eight equal parts. Seven portions of each serum were placed in small bottles and preserved by the adition of crystals of thymol. The eighth portion was used to set up agglutination tests in this laboratory. One portion of each of the preserved serums was sent to seven laboratories where they were subjected to the agglutination test for bacillary white diarrhea.

The results of these tests, which are given in Table 3, are

TABL 3.—FINDINGS OF DIFFERENT LABORATORIES

	Lab. No. 1	Lab. No. 2	Lab. No. 3	Lab. No. 4	Lab. No. 5	Lab. No. 6	Lab. No. 7	Lab. No. 8
H29	—	—	—	—	—	—	—	—
H62	—	—	—	trace	—	—		—
H71	—	—	—	—	—	—	—	—
44	+	+	+	+	+	+	+	+
F100	+	+	+	+	+	+		+
H293	+	+	+	+	+	+	+	+
H310	+	+	+	+	+	+	+	+
H413	+	+	+	+	+	+	+	+
H416	+	+	+	+	+	+	+	+
H421	+	+	+	+	+	+	+	+
H454	+	+	+	+	+	+	+	+
H472	+	+	+	+	+	+		+
H479	+	+	+	+	+	+	+	+
H484	+	+	+	+	+	+	+	+
H488	+	+	+	+	+	+	—	+
H489	±	+	+	+	+	+		+
H505	+	—	+	+	+	+	+	+
H626	+	+	+	+	+	+		+
J997	±	+	+	+	+	+		+
2471	+	+	+	+	+	+	+	+
2491	—	+	+	+	+	—		+
2495	+	+	+	+	+	+	+	+
A5903	+	+	+	+	+	+		+
A5952	—	—	—	±	—	—		±

very uniform. Laboratory No. 1 reported one sample as negative which the majority of the workers reported positive. Laboratory No. 2 also reported one sample negative which the majority found positive. Laboratory No. 3 and Laboratory No. 5 agreed with the majority report in every instance. Laboratory No. 4 reported one sample as a partial reactor which the majority of the workers reported negative. The same laboratory reported a typical trace of reaction in a sample which the majority reported negative. Laboratory No. 6 reported one sample negative which the majority of workers reported positive. This sample (2491) was the same as that reported negative by laboratory No. 1. Laboratory No. 7 only tested 14 samples of the serum, finding 10 samples unfit to test. This laboratory reported one sample negative which the majority reported positive. Laboratory No. 9 reported one sample as a partial reactor which the majority of the workers found negative. If the majority report be taken as correct in each instance the agreement of the eight laboratories for the twenty-four samples is 96.4 percent.

In justice to the investigators taking part in this work, it should be stated that the samples were not in the best of condition when shipped. The addition of thymol caused, in some instances, a heavy white precipitate which made the tests very difficult to read. In every case in which a laboratory reported a bird negative while the majority report was positive, that sample of serum was markedly cloudy so that a perfectly satisfactory reading could not be obtained.

In addition to the difficulties cited above, there is still another factor which must be considered in work of this kind. The agglutinins in fowl serum acting upon *S. pullorum* are not nearly so active after preservatives have been added to the serum. Tests with serums which had been preserved, shipped to distant points and returned to this laboratory have demonstrated this fact. So that when we consider the effect of preservatives upon the physical properties of the serum together with its effect upon the agglutinins for *S. pullorum* it may be

easily understood why discrepancies appear in such comparisons. There is no doubt whatever that could the workers taking part in this study have tested these samples of serum when fresh and unpreserved the results would have been still better. The difficulties under which they were working are shown by the fact that one laboratory found ten of the samples in such bad condition that they could not be tested.

While the number of samples tested is small the great uniformity obtained demonstrates that comparable results may be obtained with the agglutination test for the detection of *S. pullorum.*

THE CONSTANCY OF THE AGGLUTINATION TEST IN THE DETECTION OF BACILLARY WHITE DIARRHEA

Several investigators have noted that serums of hens infected with bacillary white diarrhea at times fail to agglutinate *S. pullorum.* As early as 1916, Horton[18] reported that a hen which had previously been positive to the agglutination test gave negative reactions. Erickson[19] tested 15 hens for a period of 12 months. During this time, he observed fluctuations in the reactions, some of the birds varying from positive to negative. Gwatkin[20] observed the reactions of 11 positive birds for seven months. With one exception the results were consistent. Doyle[21] performed monthly tests on 14 reacting hens. The tests covered a period of 11 months. During this time three of the birds ceased to react. Beach, Halpin and Lampman[22] conducted repeated tests on 64 hens. Of these 44 were reactors at the beginning of the experiment. The birds were tested 11 times in 13 months. Of the 44 reactors, 18 gave at least one negative test during the course of the experiment. Some of the birds gave as many as 6 or 7 negative tests. Such results caused the authors to recommend disposing of infected flocks instead of endeavoring to eliminate infected individuals.

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[18] Horton, G. D., Jour. Bact. 1, 1926, 625.
[19] Erickson, S., Missouri State Poultry Assoc. Yearbook, 1923, 31.
[20] Gwatkin, R., Report of the Ontario Veterinary College, 1924, 68.
[21] Doyle, T. M., Jour. Compar. Path. and Ther. 38, 1925, 266.
[22] Beach, B. A., Halpin, J. G., and Lampman, C. E., Jour. Amer. Vet. Med. Assoc. 70, 1927, 605.

Beach[23] at the California Station tested 70 reacting pullets at monthly intervals for 12 months. During the period which the birds were under observation 40 gave one or more negative tests. The number of negative tests in individual birds varied from one to eleven. In some instances the birds gave alternate positive and negative tests. *S. pullorum* was isolated from birds that had given three consecutive negative tests before death. *S. pullorum* was also isolated from the ovaries of birds which had never yielded a positive agglutination test. Kaupp and Dearstyne[24] tested 29 birds at monthly intervals for 11 months. During this period three birds gave negative reactions. They were also able to show that these birds laid infected eggs during the period their agglutination reaction was negative. In spite of the fluctuations observed in the tests 90 percent of the birds tested 14 months and 98 percent of the birds tested six months would have reacted at any time during the course of the investigation.

Newsom, Cross and Ufford[25] performed repeated tests on 59 birds. The length of time the birds were kept under observation varied from 12 to 24 months. The number of tests upon individual birds varied from 4 to 21. Under these conditions 13 of the birds were consistenly negative, 25 were consistenly positive, and 21 gave variable reactions. These writers concluded that the agglutination test should be applied to flocks at frequent intervals to remove all infected birds.

The results cited indicate that the agglutination test for bacillary white diarrhea may not be such an accurate method of diagnosis as it has long been thought to be. The results obtained by Beach, Halpin and Lampman[22] and Beach[23] are particularly discouraging to persons interested in the control and eradication of bacillary white diarrhea thru the application of the agglutination test.

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[23] Beach, J. R., Hilgardia, 2, 1927, 529.

[24] Kaupp, B. F., and Dearstyne, R. S., North Car. Agr. Exp. Sta. Tech. Bul., 29, 1927.

[25] Newsom, I. E., Cross, F., and Ufford, O. C., Jour. Amer. Vet. Med. Assoc., 1928, 72, 611.

Experimental Work

In order to determine the amount of variation in the agglutination test as it is conducted in this laboratory, 93 hens which had reacted to the agglutination test were collected and placed under observation. This was not a selected group, but was made up of reactors removed from farm flocks as infected individuals. The hens were placed in a house 30 feet by 15 feet and allowed to range over a large yard at all times. They were tested at monthly intervals for one year. The mortality in this group was 34.4 percent during the year they were under observation. This confirms the observation of other workers that the mortality among carriers of *S. pullorum* is very high.

The results of these tests were surprisingly consistent. During the period of observation a total of 984 tests were made on the 93 hens. Of these 984 tests only 6 were negative. These negative tests were confined to 4 birds, H197, 165, H462 H107. The records of the tests are given in Table 4. The bird H197 in table 1 is the only one giving well-defined (complete or nearly complete) agglutination at 1-40 at the beginning of the experiment that later gave a negative test. Of 874 tests performed on such birds only one negative test was obtained. The remaining five negative tests were confined to birds giving only a weak partial agglutination at 1-40 at the beginning of the experiment.

S. pullorum was not recovered from two of the four hens which gave variable reactions during the course of the experiment, altho the reactions would indicate that they were infected. It is probable that these hens were carrying the organism but it was missed on post-mortem examination.

The duration of the entire experiment was 15 months. On ten occasions every bird reacted to the test. One month, October, two birds failed to react to the test. On four of the tests: July, August, November and January, one bird failed to react each time. Expressed as percentages, for the months in which negative tests were observed, 98.8 percent of the birds reacted in July, 98.7 percent in August, 97.3 percent in October, 98.5 percent in November, and 97.9 percent in January. Viewed from this angle the agglutination test is remarkably efficient.

No attempt was made to determine the titers of the serums of the birds being tested. However, using only the two dilutions, variations in the titers of the individual hens were apparent. At times the titer of serum which caused complete agglutination at 1 to 80 would drop until only a partial agglutination at 1 to 40 would be observed. The titers of the serums of other birds increased during the period. However, it was only in the cases of the four birds previously noted that the titer became so low that no agglutination was present at 1 to 40.

During the course of the experiment 5 birds were lost. Post-mortem examinations were made of the remaining 88 hens. *S. pullorum* was recovered from 80 hens, *Eberthella sanguinarium* from 1, streptococci from 1 and in 6 cases the examination was negative.

In the hen from which *E. sanguinarium* was isolated the organism was apparently confined to the ovary. The organisms were not isolated from the heart, spleen or liver. The ova were discolored and misshapen, resembling ova infected with *S. pullorum*. This hen was in apparently good health, being killed for post-mortem examination at the end of the experiment. No other cases of fowl typhoid occurred in the flock.

Summary

The results obtained in this work, tho remarkably uniform, indicate the advisability of retesting flocks soon after the original test. This is to be especially recommended where a fairly high percentage of infected birds are found on the first test. Our opinion is that the second test should be performed after an interval of 45 to 60 days. This interval will allow incipient infections to develop to the point where they may be detected and should allow the laboratory to detect infected birds which were missed on the first test. It is obvious that the number of retests which will be required will vary with the percentage of reactors in the flock and the number of reactors found upon retesting. In formulating a program for the control of the disease the breeder should consider the possibility of having to

retest the flock and make due allowance for it. This implies that the first test should be performed at least 60 days before the start of the breeding season.

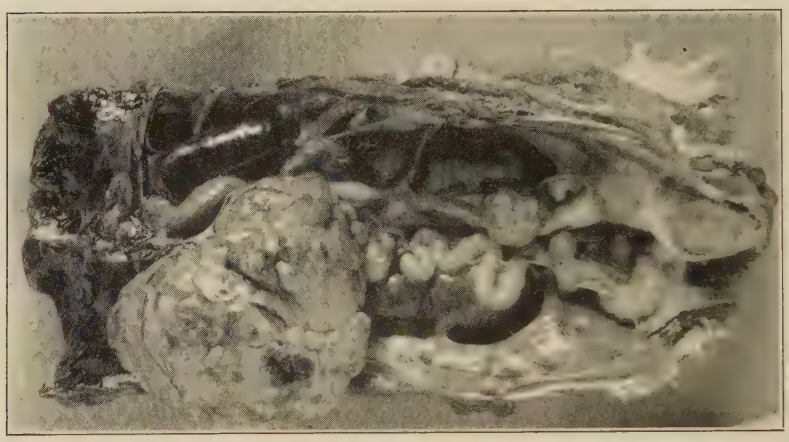

Fig. 1. Testicles and vas deferens from cockerel (J. 2319). **Salmonella pullorum** was isolated from the right testicle. Sections were made of the right testicle and the right vas deferens. The tunica albuginea was thickened. The seminiferous tubules were completely obliterated. There was no evidence of spermatozoa. Multiple small abscesses and areas showing round cell infiltration were scattered thruout the testicle. The contents of the abscesses had become inspissated. The lumen of the vas deferens was increased in size and filled with a dense, structureless, homogenous exudate. Extensive desquamation of the epithelium had taken place.

In the control of any animal disease in which biological tests are used to detect infected individuals, eradication is not expected to be accomplished thru a single application of these tests. This is especially true where the animals are inhabiting infected premises. Eradication is accomplished only thru repeated testing and strict sanitation. There is no reason to expect the agglutination test for bacillary white diarrhea to bring results that no other test can give. Because it does not do this it is sometimes severely criticized. However, this test is one of the most accurate of the biological methods at our command. Thru careful and systematic application of the agglutination test and intelligent methods of management, bacillary white diarrhea can be eradicated.

THE TRANSMISSION OF BACCILARY WHITE DIARRHEA AMONG HENS

The extent to which *S. pullorum* infection may be conveyed from hen to hen by contact is not definitely known. The control and eradication measures advocated by the majority of the

Fig. 2. Spleen from a field case of bacillary white diarrhea. S. pullorum was isolated on post-mortem. The gland was enlarged three times, due to multiple abscesses and numerous small areas of necrosis.

agencies conducting campaigns against this disease are tacit admissions that there is danger of such transmission. These measures usually include provisions for immediate removal of all reacting hens from breeding flocks. However, a review of the literature discloses that most investigators have been unable to demonstrate any transmission of the disease among hens unless male birds were present.

Rettger and Stoneburn,[26] in 1911, stated that while they had no direct evidence concerning transmission from hen to hen thru contact, they considered it improbable that infection was con-

[26] Rettger, L. F., and Stoneburn, F. H., Conn. Storrs Sta. Bul. 68, 1911.

veyed from adult to adult in this manner. One year later Rettger, Kirkpatrick and Card[27] published the result of experiments bearing on this subject. They placed seven infected hens in a pen with seven hens which were thought to be free of the disease. These hens were kept together for 22 months. Four of the non-infected hens survived thruout this period and at the end of the experiment three were found to be cariers of *S. pullorum.* This experiment was performed before the agglutination test was used for the detection of bacillary white diarrhea. The hens selected as non-infected came from a flock in which no bacillary white diarrhea was known to be present. The eggs laid by these hens were examined for a long period before the start of the experiment and *S. pullorum* was not isolated from them. While it is true that this experiment may be criticised because of the methods available at that time, it is very probable that transmission of the infection actually occurred.

Doyle[21] kept 50 hens which reacted to the agglutination test and 30 hens which did not react to the test in contact for one year. Agglutination tests performed at monthly intervals indicated that there was no transmission of infection during the course of the experiment. Bunyea[28] placed a non-reacting hen in a pen containing eleven reactors and allowed it to remain there for 76 days. At the end of this time the non-reacting hen showed no evidence of infection. Dalling, Mason and Gordon[29] state that infected hens apparently did not transmit the infection to normal hens or chicks. Brunett[30] kept 17 non-reacting hens in contact with 20 reacting hens for seven months. During this period he could observe no transmision of the disease to the non-infected hens. The agglutination test was used as means of determining infection. He concludes that "we may assume that mature hens do not ordinarily become infected by association with other mature stock." Non-infected male birds were

[27] Rettger, L. F., Kirkpatrick, W. F., and Card, L. E., Conn. Storrs Sta. Bul. 74, 1912.
[28] Bunyea, H., Jour. Amer. Vet. Med. Assoc. 70, 1927, 645.
[29] Dalling, T., Mason, J. H., and Gordon, W. S., Vet. Jour. 83, 555.
[30] Brunett, E. L., Cornell Vet. 18, 1928, 135.

placed with the hens after they had been in contact for seven months. Transmission of the disease was then observed.

Experimental Work

In the present experiment 73 hens which yielded positive reactions to the agglutination test were kept during one year in a house with 15 hens which did not react to the test. The hens were alowed to range over a large yard at all times. The dimensions of the house were 15 feet by 30 feet. The hens were trapnested in order to minimize the possibility of transmission of infection thru the ingestion of eggs containing *S. pullorum*. The non-reacting hens were obtained from a breeding flock which has been tested for bacillary white diarrhea at regular intervals for several years. All these hens had been tested on two previous occasions at intervals of six months and each time had given negative reactions to the test. They were tested a third time shortly before the beginning of the experiment and showed no evidence of being infected. These hens were two years old at the beginning of the experiment. The majority of the infected hens were also two years old, altho a few of them were three and four years old. After being placed together the hens were tested monthly. In performing the tests two dilutions were used, 1-40 and 1-80. The presence of agglutination at a dilution of 1 to 40 was considered indicative of infection. At the end of the experiment the surviving hens were slaughtered and examined for the presence of *S. pullorum*. The results of the agglutination tests and the results of the post-mortem examinations are given in table 5.

From table 5 it can be seen that eleven of the fifteen negative birds were living at the end of the experiment. Four of the hens died of intercurrent disease during the course of the investigation. None of these four hens gave a positive agglutination test while living and *S. pullorum* could not be isolated from any of them on post-mortem examination. Of the eleven hens which survived the experiment, five apparently became infected during the year. One hen became positive during the third month, one

TABLE 5.—TRANSMISSION OF S. PULLORUM INFECTION BY CONTACT

Bird No.	AGGLUTINATION TESTS Date												Post Mortem Exam.
	1 24	2 24	3 22	4 25	5 24	6 25	7 25	8 23	9 25	10 24	11 26	12 12	
H1										+	+	+ +	+
H3													—
H19													—
H49								+ +	+	+	+ +	+ +	+
H62								Dead					
H71				+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+
H81													—
H90													—
H114					Dead								—
H202								+ +	+ +	+ +	+ +	+ +	+
H204						Dead							—
H205								+ +	+ +	+ +	+ +	+ +	+
H776													—
H792													—
H105		Dead											—

−, −, no agglutination at 1-40 or 1-80.

±, −, definite but incomplete agglutination at 1-40, no agglutination at 1-80.

+, −, complete agglutination at 1-40, no agglutination at 1-80.

+ ±, complete agglutination at 1-40, definite but incomplete agglutination at 1-80.

+ +, complete agglutination at 1-40 and 1-80.

Post mortem examination

+, S. pullorum isolated.

−, S. pullorum not isolated.

during the seventh month, two during the eighth month and one during the tenth month of the experiment. *S. pullorum* was recovered from the ovaries of each of these hens. The other seven hens which survived the experiment never gave a positive test and *S. pullorum* was not isolated from them on post-mortem examination.

The foregoing facts indicate that bacillary white diarrhea is transmitted from hen to hen by contact. The negative hens used in this experiment were hatched from tested stock and no deaths due to bacillary white diarrhea occurred among the chicks. The hens were tested three times before the start of the experiment and gave negative tests each time. In view of these facts it is highly improbable that the hens were infected at the beginning of the experiment.

The results of this work confirm the earlier investigation of Rettger, Kirkpatrick and Card[27] and indicate that transmission of bacillary white diarrhea among mature hens may ocur without the presence of male birds.

FEEDING S. PULLORUM TO PULLETS AND COCKERELS

It has long been known that chickens may become infected with *S. pullorum* thru the ingestion of food containing the organism or thru the oral administration of cultures. The period of time which must elapse between the feeding of the organism and the appearance of agglutinins in the serum of the fowl is not known. This period of non-reactivity of infected birds is of importance in the application of the agglutination test. It is generally admitted that in testing a flock certain recently infected individuals may not react. If the length of time necessary for agglutinins to appear in the serum of the infected fowl could be definitely determined, the most advantageous time for retesting could be more exactly fixed.

Experimental Work

In order to determine the time necessary for agglutinin production, 16 pullets and 16 cockerels were fed cultures of *S. pul-*

lorum. The chickens used in this experiment were hatched from stock free from bacillary white diarrhea. No deaths had occurred before the beginning of the experiment. They were tested several times before the organisms were fed and no reactors were detected. Each pen received 500cc of a saline suspension of *S. pullorum,* the density of which was .25 on the MacFarland nephelometer. The birds were then 9 months old. The temperatures of the birds were taken daily for 10 days, after feeding them the organism. No significant differences in temperature were observed. The birds were tested by the agglutination test 7 days after feeding and at weekly intervals for one month. Following these weekly tests the birds were tested at monthly intervals for one year. At this time those which survived the experiment were killed and post-mortem examinations performed. All birds dying during the course of the experiment were autopsied.

Results

Following the feeding all the birds appeared to be suffering from an acute infection. They were greatly depressed, the wings and tails drooped, the feathers were ruffled, they stood about and if disturbed moved reluctantly. All were affected with a pronounced diarrhea, the fluff about the vent being greatly soiled. The egg production of the pullets was reduced to 50 percent of the pre-feeding level and remained at this low figure for three weeks. During the following three weeks egg production increased until at 6 weeks following the feeding of the organisms production reached the pre-feeding level. The period of physical depression lasted about 10 days. At this time all the birds had apparently recovered.

The results of the agglutination tests and post-mortem examinations are given in Table 6. The bacilli were fed on Feb. 17, 1928. No pre-feeding tests are included in the table since all were negative. The first test given in the table was on Feb. 24, 1928, seven days following feeding. The first 16 birds in the table are pullets, the last 16 are cockerels. Seven of the birds

TABLE 6.—RESULTS OF FEEDING A CULTURE OF S. PULLORUM TO PULLETS AND COCKERELS

Band No.	2-24-28	3-2-28	3-9-28	3-16-28	3-23-28	4-25-28	5-24-28	6-25-28	7-25-28	8-22-28	9-25-28	10-25-28	11-26-28	12-18-28	1-25-29	Post Mortem Exam.
J2306	— —	+ +	+ ±	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2309	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2310	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2312	± +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +						+
J2313	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2316	— —	— —	— —	— —	— —											—
J2329	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+
J2333	— —	— —	— —	— —	± —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2335	— —	± ±	± —	+ +												—
J2339	— —	± —	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+
J2347	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+
J2351	— —	+ +	+ +	+ +	+ +	+ +	+ +									+
J2357	± —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2372	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2388	— —	— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+
J2395	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2302	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2303	+ +	+ +	± —	+ +	+ ±	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2305	— —	— —	— —	— —	— —											—
J2315	— —	+ +	+ +	± —	± —	± ±	± ±	± ±	± —	— —	— —	— —	— —	— —	— —	—
J2318	— —	+ ±	+ +	— —	± ±	± ±	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2319	— —	— —	— —	± ±	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+
J2320	— —	+ +	+ +	+ +	± ±	± —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2321	— —	± ±	± —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2323	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2324	— —	— —	— —	— —	— —	± —	± ±	± ±	± —	— —	— —	— —	— —	— —	— —	—
J2325	+ +	+ +	+ +	± —	± —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2341	— —	+ +	± ±	± —	± ±	± ±	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2343	± ±	± ±	± —	± ±	± ±	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2344	— —	— —	— —	— —	— —											—
J2377	± —	+ +	± —	± —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—
J2385	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	—

— —, no agglutination at 1-40 or 1-80.
± —, definite but incomplete agglutination at 1-40, no agglutination at 1-80.
± —, complete agglutination at 1-40, no agglutination at 1-80.
+ ±, complete agglutination at 1-40, definite but incomplete agglutination at 1-80.
+ +, complete agglutination at 1-40 and 1-80.

Post mortem examination.
+, S. pullorum isolated.
—, S, pullorum not isolated.

became permanent reactors and *S. pullorum* was isolated from them at post-mortem. Of this number six were pullets and one was a cockerel. The bacilli were localized in the ovaries of the pullets and in the testicle and vas deferens of the cockerel. Thirteen birds gave temporary reactions which later disappeared. Three of these were pullets and ten cockerels. Post-mortem examination of these birds showed no lesions of white diarrhea nor was *S. pullorum* isolated from any of them. Whether these temporary reactions represent actual infections which were overcome without localization of the bacilli or whether they merely represent reactions to antigen administered thru the digestive tract cannot be stated. Doyle[31] has demonstrated that agglutinins may be developed in hogs by oral administration of dead cultures of *Salmonella suipestifer*. It is possible that these reactions represent the same condition. It is noteworthy that the majority, ten of thirteen, of these reactions occurred among cockerels. As noted above, only one cockerel became a permanent reactor. Probably the more pronounced of these temporary reactions represent transient infections in which permanent localization did not occur. This opinion is based on the fact that these temporary reactions were not nearly so pronounced among the pullets. There is no reason why a pullet should not react to antigen taken into digestive tract to the same degree as a cockerel, provided that actual infection does not take place in either case. The larger number of temporary reactions among the cockerels indicates that these were actual infections which were later overcome. The supposition seems reasonable that the infection did not persist among the male birds as it did among the females because the functioning ovary offers a more favorable site for permanent localization than does any organ of the cockerel. In eleven cases no agglutinins appeared in the serum at any time. Six of these birds were pullets and five were cockerels.

Agglutinins appeared in the blood of certain birds within 7 days after the feeding of *S. pullorum*. This was true of 6 of

[31] Doyle, L. P., Jour. Infect. Diseases, 42, 1928, 218.

the birds studied. At 14 days after feeding agglutinins were present in the serums of 16 of the fowls. One fowl did not react until the twenty-first day after feeding and another showed no reaction until the thirtieth day. Two fowls, J2333 and J2324, first reacted on the thirty-seventh and sixty-ninth days, respectively. These reactions were very slight and temporary. It is doubtful that they were directly connected with the feeding. One pullet reacted on the fourteenth day but failed to react again until the thirty-seventh day. Thereafter she was a permanent reactor. Many of the initial reactions were weak and might have escaped detection in routine testing. The number of bacilli administered to these birds was greater than they would ordinarily be exposed to in natural infection. This probably would bring about the formation of agglutinins in a shorter time than would natural infection. These findings indicate that in retesting flocks for recent infections and missed cases the retesting should not be done for 45 or preferably 60 days following the original test.

A FATAL INFECTION OF CHICKS DUE TO BACILLI OF THE PARATYPHOID B GROUP

Organisms of the paratyphoid B group have often been found in infections of fowls. Caged birds, such as the canary and parrot, have been infected with these organisms more often than the domesticated fowls. However, bacilli closely related to *Salmonella schottmuelleri* have been found several times in chickens. Spray and Doyle[32] described an organism of this group which was isolated from baby chicks. This bacillus was not definitely identified. Beaudette[33] and Doyle[34] have reported the presence of *Salmonella aertrycke* in diseases of chicks.

The present work is a study of organisms found in an epizootic in baby chicks. The outbreak of disease occurred in a flock of approximately 2,000 chicks. It is impossible to estimate accurately the mortality due to this disease since all the chicks that died were not examined and at the age of two weeks the

[32] Spray, R. S., and Doyle, L. P., Jour. Infect. Diseases, **28**, 1921, 43.
[33] Beaudette, F. R., Personal Communication.
[34] Doyle, T. M., Jour. Comp. Path. and Ther. **40**, 1927, 71.

chicks contracted coccidiosis. However, the mortality was approximately 25 percent before the first case of coccidiosis was detected. Paratyphoid organisms were recovered regularly from all the chicks that were examined before coccidiosis was discovered in the flock. *S. pullorum* was not found in any of the chicks.

The disease first became apparent when the chicks were three days old. The symptoms noted closely resembled those of bacillary white diarrhea. The chicks appeared dull and listless and remained near the hover of the brooder. They gradually grew weaker and died. No diarrhea was noticed. On post-mortem examination the most noticeable change was an extremely edematous condition resembling that found by Stafseth and Johnson[35] in bacillary white diarrhea. All the tissues contained an excessive amount of serous fluid and in many of the chicks there was an accumulation of 4 to 6 cc. of fluid in the abdominal cavity. In some of the chicks which died after reaching the age of 7 days small necrotic areas were noted in the liver. Occasionally petechiae were found on the epicardium. The organisms responsible for the disease were recovered consistently from the liver, heart, lungs and unabsorbed yolk. While the early age at which the disease appeared suggests that it might have been transmitted thru the egg, we believe that it was contracted after hatching. No carriers of infecting organisms could be detected among the breeding stock by the agglutination test. The breeding stock used in this flock is made up largely of the same birds each year, the only changes being occasional importations of male birds and replacement of old birds with younger stock as necessity demands. Disease due to these organisms was never recognized in the flock before the present outbreak occurred. The premises were thoroly cleaned and disinfected after the disease occurred and no further trouble from this cause has been experienced. No carriers of the organisms have been detected among the survivors of the epizootic altho a number of them have been tested by the agglutination test and

[35] Stafseth, H. J., and Johnson, E. P., Mich. Sta. Quart. Bul. 9, 1927, 155.

post-mortem examinations have been made of others. The source of infection has not been established.

The organisms isolated from the chicks were gram-negative, motile rods which did not produce indol or liquefy gelatin. Nitrate was reduced to nitrite. Lead acetate was blackened. Growth occurred in uric acid and citrate mediums. Acid and gas were produced in broth containing glucose, levulose, galactose, rhamnose, xylose, arabinose, sorbitol, dulcitol and inositol. No acid or gas was produced in broth containing lactose, sucrose, salicin, raffinose and adonite. These characters place the organisms in that division of the genus *Salmonella* generally referred to as the paratyphoid B group. After finding that the cultural characters of all the strains examined were identical only two cultures were saved for further study, the others being discarded.

Agglutinating serum was prepared for one of the cultures and the serum used in agglutination and absorption tests. It immediately became apparent that the two cultures were not antigenically identical. Agglutinating serum was then prepared for the second culture isolated from the chicks and from stock cultures of the paratyphoid B group. Using these serums comparative studies were carried out.

The designations of sources of the cultures used in the comparitive work are given below:

MC1, MC2: isolated from epizotic of chicks, 1928.

C5: stock culture of *Salmonella anatum* isolated from ducklings (Rettger and Scoville: J. Infect. Dis., 1920, 26, p. 217).

OW: *Salmonella aertrycke* isolated from ovary of breeding duck.

B: Type Binns, strain Binns.[36]

R: Type Reading, from water supply.[36] From Dr. E. O. Jordan.

N: Type Newport, from food poisoning in man. From Dr. E. O. Jordan.

S: Type Stanley, strain Stanley, from food poisoning.

[36] Schutze, H., Lancet, 1920, I, 93.

S31: *S. schottmuelleri,* from paratyphoid fever in man. From Dr. W. G. Savage.

350: *S. suipestifer,* from hog cholera. Isolated by Dr. Th. Smith, 1921.

In table 7 are recorded the agglutination reactions of the various cultures with antiserums derived from cultures MC1, MC2, OW and C5. The two cultures from chicks (MC1 and MC2) were quite different in their agglutinative characters.

TABLE 7.—AGGLUTINATION REACTIONS

	Antiserums			
Antigens	MC1	OW	MC2	C2
MC2	1000*	2000	8000	8000
C5	1000	2000	8000	8000
MC1	8000	8000	2000	2000
OW	8000	8000	2000	2000
B	8000	8000	1000	2000
R	400	1000	2000	4000
N	1000	2000	2000	2000
S	1000	2000	1000	2000
S31	2000	4000	400	2000
350	2000	2000	1000	4000

*Figures indicate highest dilution at which agglutination occurred.

The antiserum derived from MC1 agglutinated the homologous organism, OW and B in high dilution, while the other cultures were acted upon only in lower dilutions. The antiserum derived from MC2 agglutinated the homologous organism and the culture of *S. anatum,* C5, in high dilution while the other strains tested were agglutinated only in the lower dilutions. The reactions obtained with C5 antiserum closely resembled those obtained with MC2 antiserum. The antiserum derived from OW gave reactions similar to MC1 antiserum. The agglutination tests indicate that MC1 is closely related to *S. aertrycke* while MC2 is apparently identical with *S. anatum.*

The results obtained in agglutinin absorption tests confirm the relations indicated by the agglutination tests. Strain OW is able to effect a complete removal of agglutinins from MC1 antiserum and MC1 removes from OW antiserum all agglutinins acting upon the homologous organism. None of the other organ-

isms tested were able to remove agglutinins completely from these serums. Strain B, a representative of the Binns type, reduced the titer of the serums approximately 90 percent Absorption of the serums with increased amounts of this organism did not lead to a further removal of the agglutinins. White[37] has stated that the Binns type is probably a non-specific race of *S. aertrycke*. The results obtained here indicate their close relationship. Absorption of these serums with the remaining organisms had little effect on their titer for the homologous strains.

All the agglutinins acting upon the serum strain are removed from MC2 antiserum by C5. Likewise MC2 is able to remove agglutinins completely from C5 antiserum. None of the other organisms used in the tests were able to lower materially the titer of the serums for the homologous organisms. It is evident that at least two types of paratyphoid organisms were responsible for the outbreak of disease studied.

The recovery of two types of paratyphoid organisms from a single outbreak of disease has often been reported. Ten Broeck[38] found two types of paratyphoid bacilli in swine. Rettger and Scoville[39] isolated a number of cultures of paratyphoid bacilli from ducklings. These were later shown to constitute two distinct types. Amoss and Hasselbauer[40] have isolated *S. enteritidis* and *S. aertrycke* from the same outbreak of disease in mice. Nelson[41] noted two types of paratyphoid bacilli in an epizotic of guineapigs. Friedlander[42] found two distinct types of the paratyphoid B group in cats.

One of the organisms isolated from the chicks, *S. aertrycke*, is widespread in nature. It has been found in infections of chicks by other workers.[33] [34] The second type isolated from the outbreak, *S. anatum*, has never before been found in chickens. It was first described by Rettger and Scoville.[39] The strains

[37] White, P. B., Med. Res. Council, Spec. Rpt. Series No. 91.
[38] Ten Broeck, C., Jour. Exp. Med., **28**, 1918, 759.
[39] Rettger, L. F., and Scoville, M., Jour. Infect. Diseases, **26**, 1920, 217.
[40] Amoss, H., and Hasselbauer, P., Jour. Exp. Med., **36**, 1923, 541.
[41] Nelson, J. B., Jour. Exp. Med., **46**, 1927, 541.
[42] Friedlander, R. D., Jour. Amer. Vet. Med. Assoc., **74**, 1929, 932.

which they isolated from ducklings were later found by Cooper and Krumwiede[43] and Edwards and Rettger[44] to be divisible into two types, one identical with *S. aertrycke,* the other unlike any of the cultures of the paratyphoid group with which it was compared. Until now the occurrence of this independent type of paratyphoid, here referred to as *S. anatum,* has not been recorded since its original isolation from ducklings. It is interesting that the organism should be found in a location far removed from the original site of isolation and that it was associated with the same organism. *S. aertrycke,* in both instances. The reisolation of this organism lends added weight to its importance as a member of the paratyphoid B group.

Summary

An epizootic of chicks has been observed in which the mortality was approximately 25 percent. Two organisms of the paratyphoid B group, *S. aertrycke* and *S. Anatum,* were isolated from the chicks examined.

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[43] Cooper, G. M., and Krumwiede, C., Abstr. Bact., 8, 1924, 25.
[44] Edwards, P. R., and Rettger, L. F., Abstr. Bact., 8, 1924, 25: Jour. Bact. 13, 1927, 73.